

Effects of Chikungunya virus titers in blood meals on virus infection, dissemination, and transmission in Asian tiger mosquito: *Aedes albopictus* (Diptera: Culicidae)

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Abstract

Chikungunya virus (CHIKV) is an important mosquito-borne virus and transmission cycle of this virus involves mosquito vectors and infected vertebrate hosts. However, the study about vector competence for CHIKV in Thailand is limited. This study was conducted to examine the effects of CHIKV titers in blood meals on vector competence of *Aedes albopictus* (Diptera: Culicidae). Five groups of *Ae. albopictus* were allowed to feed on different levels of CHIKV in the blood meals which were 10^2 , 10^3 , 10^4 , 10^5 , and 10^6 CID₅₀/ml of CHIKV. Body, legs and wings, and saliva samples from blood-fed mosquitoes were assayed for the presence of CHIKV by using immunocytochemistry staining on day 14 post blood feeding. Percent virus infection, dissemination, and transmission is defined as percent of blood-fed mosquitoes with virus in their bodies, legs and wings, and saliva, respectively. The percent infections were 83.3, 90, 100, 100, and 100%, the percent disseminations were 70.8, 86.7, 100, 90, and 98%, and the percent transmissions were 41.6, 70, 100, 90, and 82.4% after having been fed on 10^2 , 10^3 , 10^4 , 10^5 , and 10^6 CID₅₀/ml of CHIKV, respectively. However, there was no significant difference of the percent transmission after having been fed on 10^4 and 10^5 CID₅₀/ml of CHIKV. This study suggested that *Ae. albopictus* are susceptible for CHIKV infection and efficient vectors for CHIKV transmission, and CHIKV titers in blood meals have effects on virus infection, dissemination, and transmission in *Ae. albopictus* or vector competence of this mosquito.

Keywords: *Aedes albopictus*, Chikungunya virus, Transmission, Vector competence

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Introduction

Chikungunya virus (CHIKV) is an emerging or re-emerging mosquito-borne virus belonging to the genus *Alphavirus* of the family *Togaviridae*. This virus has an antigenic relationship with Mayaro, O'nyong-nyong, and Semliki virus. It is an enveloped, single-stranded, positive-sense RNA virus. It was first discovered in Tanzania in 1952 and was first identified in Thailand in 1958 (Halstead et al., 1969). CHIKV can be classified into four lineages: West Africa, East Central and South Africa, Asian, and Indian Ocean lineage. Recently, the emerging and re-emerging of this virus have occurred in several countries including Thailand (Lam et al., 2001; Chusri et al., 2011; Duong et al., 2012). The Asian lineage was responsible for the previous outbreak, whereas the Indian Ocean lineage (IOL) contributed to the current outbreak in Thailand (Theamboonlers et al., 2009). CHIKV causes high morbidity but low mortality in humans. A large number of CHIKV-infected travelers have been identified after traveling to endemic countries (Pile et al., 1999; Hochedez et al., 2006; Gosciminski et al., 2015; Hwang and Lee, 2015; Kondo et al., 2016; Imai et al., 2016). The present situation of international travel poses a new challenge that demands increased awareness of the possibility of emerging infectious diseases (Tanay et al., 2008; Tanay, 2016). Symptoms of infected patients including acute febrile illness, headache, muscle pain, joint pain, joint swelling, and rash usually begin 3-7 days after being bitten by infected mosquitoes (Suryawanshi et al., 2009; Theamboonlers et al., 2009; Valampampil et al., 2009). The study by Wattanaveeradej et al. (2006) indicated that 33.6% of infants at delivery from March 1998 to October 1999 at the Phramongkutklao Hospital, Bangkok, Thailand had antibodies to CHIKV and the half-life of antibody persisted for 35.5 days.

Transmission cycle of CHIKV involves infected vertebrate hosts and mosquito vectors. Different mosquito species play important roles as potential vectors for CHIKV in different areas and countries (Pages et al., 2009; Richards et al., 2010; van den Hurk et al., 2010; McTighe and Vaidyanathan, 2012; Richard et al., 2016). Nevertheless, *Aedes aegypti* and *Ae. albopictus* are principal mosquito vectors for the transmission of CHIKV (Banerjee et al., 1988; Vega-Rua et al., 2014; Diaz-Gonzalez et al., 2015). CHIKV was detected in both sexes of *Ae. aegypti* and *Ae. albopictus* in Thailand. However, the infection rate in *Ae. albopictus* was higher than that in *Ae. aegypti*. The emergence of CHIKV in adult male mosquitoes of both species indicated a role of transovarial transmission of the CHIKV in field population of the mosquito vectors in Thailand (Thavara et al., 2009). A laboratory study also demonstrated vertical transmission of CHIKV in *Ae. aegypti* and *Ae. albopictus* mosquitoes in Thailand (Chompoonsri et al., 2016). The study by Jain et al. (2016) documented CHIKV infection in mosquito larvae, pupae, and progeny of adults collected from a field in India, which revealed the presence of vertical transmission of CHIKV in field population of *Ae. aegypti*.

Ae. albopictus or Asian tiger mosquito is the invasive bridge vectors for zoonotic viruses found in

various geographical areas. These mosquitoes were originally found in Asia and introduced into Africa, America, Australia, and Europe (Akiner et al., 2016). They have exophagic and exophilic behaviors, and opportunistic feeding behaviors on a wide range of hosts. *Ae. albopictus* is concerned with various vertebrate hosts and virus ecology and can pose higher risks of spreading arboviruses to human population (Delatte et al., 2010). There were few studies of the mosquito infection, dissemination, and transmission with CHIKV strain responsible for the current outbreak in Thailand. More studies need to be performed to address the role and relationship between mosquitoes and CHIKV. Therefore, this study was conducted to examine the vector competence of *Ae. albopictus*, and the effects of CHIKV titers in blood meals on virus infection, dissemination, and transmission in *Ae. albopictus*.

Materials and Methods

Mosquito and mosquito infection: Laboratory-reared *Aedes albopictus* was used in this study. Originally collected from Nonthaburi province and colonized by the Department of Medical Sciences, Ministry of Public Health, Thailand, the mosquitoes have been maintained in the insectary, Parasitology Unit, Department of Veterinary Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand for more than 10 generations. Conditions for mosquito rearing in the insectary were 25°C and 80 ± 5% relative humidity with 12:12 hr light/dark cycle. The mosquitoes were allowed to feed on 10% sucrose *ad libitum*.

Five- to 10-day-old mosquitoes were allowed to feed on different levels of CHIKV (Thailand 2010 strain) in blood meal by using an artificial membrane feeder. Feeding success rates of the mosquitoes and different membranes on the artificial membrane feeder were compared and identified in our previous study (unpublished data). The feeding success rate of the mosquitoes through fresh pork intestine is higher than that through plastic paraffin film (Parafilm M, Pechiney plastic packaging, Chicago, IL). For this reason, fresh pork intestine was used as the feeding membrane in the present study.

Virus and cell culture: Thailand 2010 strain of Chikungunya virus (CHIKV) was used in this study. CHIKV was originally isolated from an infected patient during the outbreak in Narathiwat province, southern Thailand in 2010. It was supplied by the Faculty of Medicine, Chulalongkorn University, Thailand. CHIKV was propagated and assayed in Vero-76 cells. Molecular identification of the virus was conducted by reverse transcription polymerase chain reaction (RT-PCR) and direct sequencing. It was in the Indian Ocean lineage (IOL) with an alanine-to-valine substitution at the position 226 of the E1 envelope glycoprotein (E1-A226V).

Virus assay: CHIKV in the mosquito blood meals and mosquito samples were examined through immunocytochemistry staining and confirmed by reverse transcription polymerase chain reaction.

Immunocytochemistry (ICC) staining: CHIKV in the mosquito blood meals and mosquito samples were examined. Ten-fold dilution of blood meal samples were assayed for an amount of the virus in Vero-76 cells through ICC staining. Virus titers of CHIKV in the mosquito blood meals were expressed as CID_{50}/ml (Reed and Muench, 1938). CHIKV infection in bodies, legs and wings, and saliva collected from tested mosquitoes was assayed for presence of the virus in Vero-76 cells by means of ICC staining.

The samples were inoculated in a confluent monolayer of Vero-76 cells in a 96-well plate for three days. The inoculated cells were then fixed with 4% formaldehyde at room temperature for 25 min and washed three times with 0.5% tween in Phosphate Buffer Saline (PBS). Mouse monoclonal antibody to CHIKV (abcam®, USA) was added, incubated at room temperature for two hr, and washed three times with 0.5% tween in PBS. Polyclonal rabbit anti-mouse immunoglobulins/HRP (Dako, Denmark) was added, incubated at room temperature for two hr, and washed three times with 0.5% tween in PBS. AEC (3-amino-9-ethylcarbazole) substrate (Sigma-Aldrich, USA) was added and incubated at room temperature for one hr. The plate was then washed with distilled water, dried at room temperature, and examined under a light microscope. Single measurement with four wells of inoculated cells was used for virus titer examination through ICC staining.

Reverse transcription polymerase chain reaction (RT-PCR): CHIKV in the mosquito blood meals and mosquito samples were confirmed by RT-PCR. Viral nucleic acid was extracted from the cell culture media sample through viral nucleic acid extraction kit II (Geneaid, Taiwan) following the manufacturer's instructions. The extracted nucleic acid was kept at $-80^{\circ}C$ until tested. Each extracted viral nucleic acid sample was tested for CHIKV by means of RT-PCR, which was slightly modified from that of CV et al. (2007) and Theamboonlers et al. (2009). The primers used in this study were DVRChk-Reverse 5'GGGCGGGTAGTCCATGTTGTAGA3' and DVRC hk-Forward 5'ACCGGCGTCTACCCATTCATG T3' (CV et al., 2007). The PCR product was analyzed in 2% agarose gel with an expected 330 base pair band.

Experimental design and data analysis: Five groups of five- to 10-day-old mosquitoes were allowed to feed on different levels of CHIKV (Thailand 2010 strain) in blood meal by using an artificial membrane feeder. The mosquitoes were starved from 10% sucrose for 24 hr before blood feeding. Swine intestines were cleaned several times with distilled water before use. Five ml of blood meal which was composed of 0.5 ml of stock CHIKV, 3 ml of clean sheep blood, 1 ml of fetal bovine serum (FBS) (Gibco™, Thermo Fisher Scientific, USA), and 0.5 ml of 1% sucrose was added in the artificial membrane feeder. Temperature of the blood meal in the feeder was maintained at $37^{\circ}C$ by warm water circulation in the outer part of the feeder. Each mosquito group (60 mosquitoes) was then allowed to feed for 45-60 min and the engorged mosquitoes were maintained in the insectary at $25^{\circ}C$ and $80 \pm 5\%$ relative humidity with

12:12 hr light/dark cycle for 14 days. Non-blood-fed, partially blood-fed, and dead mosquitoes were removed from the study.

In this study, percent infection was defined as percent of blood-fed mosquitoes with virus in bodies. Percent dissemination was defined as percent of blood-fed mosquitoes with virus in hemocoel as indicated by detecting virus in legs and wings. Percent transmission was defined as percent of blood-fed mosquitoes with virus in saliva (Tiawsirisup et al., 2004, 2005). On day 14 post blood feeding (PBF), each CHIKV blood-fed mosquito's body, legs and wings, and saliva were assayed for the presence of CHIKV.

The mosquitoes were anesthetized at $-20^{\circ}C$ and the mosquito legs and wings were removed and kept in 500 μl of Minimum Essential Media (MEM) (Gibco™, Thermo Fisher Scientific, USA). For mosquito saliva collection, the mosquito was then allowed to feed on 20 μl of 20% FBS in MEM in a capillary tube for 20 min. Each saliva sample was transferred into a separate tube containing 200 μl of 20% FBS in MEM. The body, and legs and wings from an individual mosquito were ground separately in a tube containing 500 μl of 20% FBS in MEM, and filtered through 0.45 μm membrane filter before being passed into 96-well plates containing a confluent monolayer of Vero-76 cells. The saliva sample was passed directly into a confluent monolayer of Vero-76 cells without filtering. Inoculated cells were observed for cytopathic effect (CPE) for up to four days, examined through ICC staining, and confirmed by RT-PCR.

Statistical analysis: Differences in percent infection, dissemination, and transmission among different levels of virus in the blood meal which were 10^2 , 10^3 , 10^4 , 10^5 , and 10^6 CID_{50}/ml of CHIKV were compared by t-test.

Results

The vector competence of *Aedes albopictus* for the Chikungunya virus (CHIKV) and the effects of CHIKV titers in blood meal on virus infection, dissemination, and transmission in *Ae. albopictus* were examined in this study. Five groups of *Ae. albopictus* were allowed to feed on different levels of Thailand 2010 strain CHIKV in the blood meal which were 10^2 , 10^3 , 10^4 , 10^5 , or 10^6 CID_{50}/ml of CHIKV. On day 14 post blood feeding (PBF), the body, leg and wing, and saliva samples from the blood-fed mosquitoes were assayed for the presence of CHIKV through immunocytochemistry (ICC) staining as indicated by red brown color in the cells. Culture media from the infected cells were also confirmed by reverse transcription polymerase chain reaction (RT-PCR).

The percent CHIKV infection in *Ae. albopictus* was 83.3% and increased to 90% after being fed on 10^2 and 10^3 CID_{50}/ml of CHIKV, respectively. However, there was no significant difference in the percent infection between these two CHIKV levels. After blood meals with the titers of 10^4 CID_{50}/ml of CHIKV and higher, all blood-fed mosquitoes were infected with CHIKV. The percent CHIKV dissemination in *Ae. albopictus* was 70.8% after being fed on 10^2 CID_{50}/ml of

CHIKV, and increased to 86.7, 100, 90, and 98% after blood meals with the titers of 10^3 , 10^4 , 10^5 , and 10^6 CID₅₀/ml of CHIKV, respectively. However, there was no significant difference in the percent CHIKV dissemination among the virus titers of 10^3 CID₅₀/ml of CHIKV and higher. The percent CHIKV transmission in *Ae. albopictus* was 41.6% after being fed on 10^2 CID₅₀/ml of CHIKV, and increased to 70, 100, 90, and 82.4% after blood meals with the titers of 10^3 , 10^4 , 10^5 , and 10^6 CID₅₀/ml of CHIKV, respectively. There were significant differences in the percent

CHIKV transmission among the different virus titers of CHIKV (Table 1). The lowest percent transmission was 41.6% and the highest percent transmission was 100% after being fed on 10^2 and 10^4 CID₅₀/ml of CHIKV, respectively. The percent transmission after being fed on 10^2 CID₅₀/ml was significantly different from that after being fed on 10^3 , 10^4 , 10^5 , and 10^6 CID₅₀/ml while the percent transmission after being fed on 10^4 CID₅₀/ml was significantly different from that after being fed on 10^2 , 10^3 , and 10^6 CID₅₀/ml of CHIKV.

Table 1 Percent infection, dissemination, and transmission of Chikungunya virus (CHIKV) in *Aedes albopictus* 14 days after feeding on CHIKV infected blood meal

CHIKV titer in mosquito blood meal ^a	No. of tested mosquitoes	Percent infection ^b	Percent dissemination ^c	Percent transmission ^d
2	24	83.3 ¹	70.8 ¹	41.6 ¹
3	30	90 ¹	86.7 ²	70 ²
4	30	100 ²	100 ²	100 ³
5	30	100 ²	90 ²	90 ^{3,4}
6	51	100 ²	98 ²	82.4 ^{2,4}

^aTiter is expressed as log₁₀ CID₅₀/ml.

^bPercent infection is defined as percent of blood-fed mosquitoes with virus in bodies.

^cPercent dissemination is defined as percent of blood-fed mosquitoes with virus in hemocoel as indicated by detecting virus in legs and wings.

^dPercent transmission is defined as percent of blood-fed mosquitoes with virus in saliva.

Values within each category (percent infection, percent dissemination, and percent transmission) that have a numerical superscript letter in common indicate no statistically significant differences.

Discussion

Aedes albopictus can be found throughout Thailand, particularly in rural areas. They are competent vectors for different arboviruses, including Chikungunya (CHIK), Dengue, West Nile (WN), and Zika viruses (Tiawsirisup et al., 2004, 2005; Vega-Rua et al., 2013; Akiner et al., 2016; Tilak et al., 2016). However, the study of mosquito vector competence for CHIKV in Thailand is limited. This study was, therefore, conducted to examine the vector competence of *Ae. albopictus* for CHIKV, and the effects of CHIKV titers in blood meals on virus infection, dissemination, and transmission in *Ae. albopictus*.

CHIKV used in this study was isolated from a patient during the outbreak of this virus in Thailand in 2010, and it was propagated in the laboratory. It is in the Indian Ocean lineage (IOL) with an alanine-to-valine substitution at the position 226 of the E1 envelope glycoprotein, which is in the same lineage as the 2008 Thailand strain. The genome sequences of CHIKV isolated from the outbreak in 2008 in Thailand are related to the strains isolated from the outbreaks in 2007 in India and in 2008 in Singapore, but different from the virus isolated in 1988 and during 1995-1996 in Thailand (Theamboonlers et al., 2009).

A study of mosquito vector competence of CHIKV indicated that the mosquito species which were responsible for the current outbreak included *Ae. albopictus*, whereas *Ae. aegypti* contributed to the previous outbreak in Thailand (Thavara et al., 2009). Tsetsarkin et al. (2007) also affirmed that *Ae. albopictus* was the more potential vector for CHIKV than *Ae. aegypti* due to the mutation of the virus which allowed them to adapt to different mosquito vectors from the past. Vertical transmission in mosquitoes may contribute to the maintenance of CHIKV in nature. Chompoonsri et al. (2016) demonstrated that *Ae. aegypti*

and *Ae. albopictus* mosquitoes from Thailand were capable of transmitting the Indian Ocean lineage of CHIKV vertically in the laboratory. They also showed that *Ae. albopictus* was more susceptible to CHIKV and had a greater ability to transmit the virus vertically than *Ae. aegypti*. However, Wong et al. (2016) investigated vertical transmission of infectious clones of CHIKV in *Ae. aegypti* from Malaysia in laboratory experiment. Eggs and adult progeny from the second gonotrophic cycles of infected parental mosquitoes were tested by RT-PCR. There was detectable CHIKV RNA in 56.3% of the pooled eggs and 10% of the adult progeny, but there was no detectable infectious virus through plaque assay.

In the present study, the blood-fed mosquitoes were examined for the presence of CHIKV in different parts of mosquitoes on day 14 post blood feeding (PBF). The percent CHIKV infections in *Ae. albopictus* were 83.3, 90, 100, 100, and 100% after being fed on 10^2 , 10^3 , 10^4 , 10^5 , and 10^6 CID₅₀/ml of CHIKV, respectively. The percent CHIKV disseminations in *Ae. albopictus* were 70.8, 86.7, 100, 90, and 98% and the percent CHIKV transmissions in *Ae. albopictus* were 41.6, 70, 100, 90, and 82.4% after blood meals with the titers of 10^2 , 10^3 , 10^4 , 10^5 , and 10^6 CID₅₀/ml of CHIKV, respectively. More studies need to be performed of the virus infection, dissemination, and transmission in *Ae. albopictus* after taking blood meals with virus titers less than 10^2 CID₅₀/ml of CHIKV to indicate the minimum infectious dose of CHIKV in this mosquito. Low CHIKV titers can usually be found in infected animals in nature and laboratory animals.

The percent virus infection, dissemination, and transmission were lowest and highest after being fed on 10^2 and 10^4 CID₅₀/ml of CHIKV, respectively. However, there was no significant difference among the percent infections after being fed on 10^4 , 10^5 , and

10^6 $\text{CID}_{50}/\text{ml}$ and there was no significant difference between the percent transmissions after being fed on 10^4 and 10^5 $\text{CID}_{50}/\text{ml}$ of CHIKV. The lowest percent transmission was 41.6% and the highest percent transmission was 100% after being fed on 10^2 and 10^4 $\text{CID}_{50}/\text{ml}$ of CHIKV, respectively. The high virus titer in mosquito blood meal might cause high mortality in the blood-fed mosquitoes and affect the average percent transmission. The difference in mosquito intrinsic factors among each mosquito might also affect the virus infection, dissemination, and transmission. The present study indicated that the CHIKV transmission by infected *Ae. albopictus* occurred after blood meals with the titer of 10^2 $\text{CID}_{50}/\text{ml}$, which is the titer that can be found in human and various animals. *Ae. albopictus* is susceptible to CHIKV infection and is the efficient vector for CHIKV transmission. Also, CHIKV titers in blood meals have effects on virus infection, dissemination, and transmission in *Ae. albopictus*. These mosquitoes play important roles in the ecology of CHIKV, therefore mosquito control must be concerned during the outbreak of this virus.

The blood-fed mosquitoes were tested on day 14 PBF because it is the optimal day for examination of virus infection, dissemination, and transmission in mosquito vectors as described in other studies (Tiawsirisup et al., 2004, 2005; Erickson et al., 2006; Tiawsirisup et al., 2008). CHIKV susceptibility varies by virus strain, and mosquito species and strain. The Asian strain of CHIKV starts to replicate at 5-6 days post infection (DPI) with the maximum virus yield at 5-10 DPI in both *Ae. aegypti* and *Ae. albopictus*. The variant Central/East/South African (CESA) virus genotype replicates earlier at 1 DPI with the maximum virus yield at 3-6 DPI in *Ae. albopictus* females while the nonvariant virus strain replicates at 1-2 DPI with the maximum virus yield at 6-12 DPI. In *Ae. aegypti*, these viruses replicate at 1-2 DPI, with maximum yields at 4-5 DPI (Chen et al., 2015).

In this study, the lowest virus titer in the blood meal was 10^2 $\text{CID}_{50}/\text{ml}$ and the percent infection was 83.3%, which is very high when compared with the percent infection of WNV in *Ae. albopictus* (Tiawsirisup et al., 2004). The percent WNV infections in *Ae. albopictus* were 0, 0, 89, 98, 93, 91, and 90% after being fed on $10^{2.5}$, 10^5 , 10^7 , $10^{7.5}$, 10^8 , $10^{8.5}$, and $10^{9.5}$ $\text{CID}_{50}/\text{ml}$ of WNV, respectively. Even though the percent CHIKV infection was 83.3%, the percent dissemination and transmission were 70.8% and 41.6%, respectively. These findings indicate that there were some degrees of virus barrier in the mosquitoes which were infection, dissemination, and transmission barriers. These barriers were involved in the replication of the virus in the mosquitoes as indicated in other previous studies (Tchankouo-Nguetcheu et al., 2010; Darwin et al., 2011; Arias-Goeta et al., 2013). The present study suggests that *Ae. albopictus* is susceptible to CHIKV infection and is the efficient vector for CHIKV transmission. CHIKV titers in blood meals also affect virus infection, dissemination, and transmission in *Ae. albopictus* or vector competence of this mosquito. The information in this study will be useful for the understanding of the ecology of CHIKV in nature in Thailand and also for disease surveillance, vector

control, and prevention of CHIKV outbreak in Thailand.

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บทคัดย่อ

ผลของปริมาณเชื้อไวรัสชิคุนกุนยาในเลือดต่อการติดเชื้อ การกระจายเชื้อ และการถ่ายทอดเชื้อในยุ้งลายสวน

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เชื้อไวรัสชิคุนกุนยาเป็นเชื้อที่มีเยื่อเป็นแมลงพาหะในการนำเชื้อ วงจรการถ่ายทอดเชื้อจะเกี่ยวข้องกับยุงพาหะนำเชื้อและโฮสต์ที่มีการติดเชื้อ อย่างไรก็ตามการศึกษาเกี่ยวกับศักยภาพของยุงพาหะนำเชื้อไวรัสชิคุนกุนยาในประเทศไทยนั้นไม่มีมากนัก งานวิจัยนี้ได้ทำการศึกษาผลของปริมาณเชื้อไวรัสชิคุนกุนยาในเลือดที่ยุ้งลายสวนกินเข้าไปต่อศักยภาพของยุงในการนำเชื้อ การศึกษานี้ประกอบไปด้วยยุ้งลายสวนจำนวน 5 กลุ่ม ซึ่งได้รับเชื้อไวรัสชิคุนกุนยาในปริมาณที่แตกต่างกัน ได้แก่ 10^2 , 10^3 , 10^4 , 10^5 และ 10^6 $\text{CID}_{50}/\text{ml}$ หลังจากนั้น 14 วัน ทำการศึกษาการติดเชื้อไวรัสในส่วนของลำตัว ขาและปีก และในน้ำลายของยุง โดยวิธี immunocytochemistry staining ร้อยละของการติดเชื้อ การกระจายเชื้อ และการถ่ายทอดเชื้อในยุ้งประเมินจากร้อยละของการติดเชื้อในส่วนของลำตัว ขาและปีก และในน้ำลายของยุงที่ได้รับเชื้อเข้าไป ตามลำดับ การศึกษานี้พบว่า การติดเชื้อในยุ้งมีค่าร้อยละ 83.3, 90, 100, 100 และ 100 การกระจายของเชื้อในยุ้งมีค่าร้อยละ 70.8, 86.7, 100, 90 และ 98 และการถ่ายทอดเชื้อในยุ้งมีค่าร้อยละ 41.6, 70, 100, 90 และ 82.4 หลังจากที่ได้รับเชื้อปริมาณ 10^2 , 10^3 , 10^4 , 10^5 และ 10^6 $\text{CID}_{50}/\text{ml}$ ตามลำดับ อย่างไรก็ตาม ไม่พบความแตกต่างอย่างมีนัยสำคัญระหว่างการถ่ายทอดเชื้อในยุ้งหลังจากที่ได้รับเชื้อปริมาณ 10^4 และ 10^5 $\text{CID}_{50}/\text{ml}$ การศึกษานี้บ่งชี้ว่า ยุ้งลายสวนเป็นยุงที่มีความไวต่อการติดเชื้อไวรัสชิคุนกุนยา และเป็นยุงที่มีศักยภาพในการถ่ายทอดเชื้อไวรัสชิคุนกุนยา รวมทั้งพบว่า ปริมาณของเชื้อในเลือดที่ยุ้งกินเข้าไปนั้นมีผลต่อการติดเชื้อ การกระจายเชื้อ และการถ่ายทอดเชื้อในยุ้งลายสวน หรือมีผลต่อศักยภาพของยุ้งลายสวนในการนำเชื้อไวรัสชิคุนกุนยา

คำสำคัญ: ยุ้งลายสวน เชื้อไวรัสชิคุนกุนยา การถ่ายทอดเชื้อ ศักยภาพในการนำเชื้อ

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